JOURNAL OF ANIMAL SCIENCE

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J Anim Sci 2009.87:1184-1191.
doi: 10.2527/jas.2008-1258 originally published online Nov 7, 2008;

Influence of toxic endophyte-infected fescue on sperm characteristics and endocrine factors of yearling Brahman-influenced bulls[1,2]

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ABSTRACT: Sixteen (mean age = 1.1 ± 0.1 yr; mean BW = 478 ± 34 kg) Brahman-influenced bulls were used to determine the influence of fescue type on sperm characteristics and serum concentrations of prolactin, cortisol, and testosterone. Bulls were blocked by BW, scrotal circumference (SC), and pregrazing sperm characteristics and randomly assigned to graze toxic endophyte-infected (EI; 4 bulls/pasture; 2 pastures) or novel endophyte-infected (NE; 4 bulls/pasture; 2 pastures) tall fescue for 121 d. Semen was collected by electroejaculation, and SC was measured and blood samples collected monthly. Sperm were evaluated for motility and morphology with an integrated visual optical system. Overall mean concentration of prolactin was decreased more ($P < 0.01$) in EI bulls than NE bulls from May to August. Scrotal circumference was not affected by fescue type ($P = 0.58$); overall SC averaged 36.7 ± 2.3 cm. Percentage of live sperm was not different ($P = 0.24$) between NE bulls (80%) than EI bulls (67%) in July and August. Bulls grazing NE fescue had more ($P < 0.06$) motile sperm than EI bulls in July and August. Percentages of progressive (57 vs. 38%, NE and EI, respectively; $P < 0.06$) and rapid (67 vs. 46%, NE and EI, respectively; $P = 0.04$) sperm were greater from bulls grazing NE than EI bulls in July and August. Average velocity of the smoothed sperm path and progressive velocity in a straight line from the beginning to the end of the sperm track were slower ($P < 0.09$) in EI bulls than NE bulls and were slower ($P = 0.04$) in August compared with July. Mean width of head oscillation as the sperm swims was less ($P < 0.06$) in August than July. Concentrations of cortisol and testosterone were not ($P > 0.10$) influenced by fescue type. Semen from bulls grazing EI had reduced motility and morphology than bulls grazing NE. Detrimental effects of toxic fescue may not be mediated by cortisol, testosterone, or both. Semen quality of bulls grazing toxic EI tall fescue was decreased with increased maximum ambient temperatures.

Key words: bull, fescue, integrated visual optical system, sperm

J. Anim. Sci. 2009. 87:1184–1191
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INTRODUCTION

Consumption of toxic endophyte-infected [**EI**; *Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorus arundinaceum* (Schreb.) Dumort.] tall fescue decreases reproductive performance of cows (Browning et al., 1998a; Burke et al., 2001; Jones et al., 2003). However, minimal research exists of the negative effects of toxic tall fescue on bull reproductive performance. Ergot alkaloids, compounds that mediate fescue toxicosis, inhibited sperm motility (Wang, 2006) and decreased the ability of sperm to fertilize oocytes (Schuenemann et al., 2005b). Further, SC was decreased and scrotal temperature increased in Angus bulls fed toxic fescue seed diets (Jones et al., 2004). A novel endophyte-infected

[1]Names are necessary to report factually on available data; however, the USDA does not guarantee or warrant the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that also may be suitable.

[2]This study was supported in part by USDA-ARS cooperative agreement 58-6227-8-040. The authors gratefully acknowledge S. Tabler, B. Woolley, and L. Huddleston, USDA-ARS, for technical assistance and daily animal management; and D. Burner, USDA-ARS, for ambient temperature measurements. Appreciation is expressed to A. F. Parlow (National Hormone and Peptide Program) for supplying materials used in the prolactin RIA.

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Received June 23, 2008.
Accepted October 29, 2008.

Table 1. Sperm variables measured by the Hamilton-Thorne Sperm Analyzer (Hamilton-Thorne Biosciences, Beverly, MA)

Variable	Description
Motile	% of total sperm moving at path velocity ≥30 µm/sec and progressive velocity ≥15 µm/sec
Progressive	% of total sperm moving at path velocity ≥50 µm/sec and straightness ≥70%
Rapid	Progressive % with path velocity >50 µm/sec
Medium	Progressive % with path velocity <50 µm/sec but >30 µm/sec
Slow	% of total sperm moving at path velocity <30 µm/sec and progressive velocity <15 µm/sec
Static	Sperm not moving at all
Path velocity (VAP)	Average velocity of the smoothed cell path (µm/sec)
Progressive velocity (VSL)	Average velocity measured in a straight line from the beginning to the end of track
Track speed (VCL)	Average velocity measured over the actual point-to-point track
Lateral amplitude	Mean width of the head oscillation as the sperm swims
Beat frequency	Frequency of sperm head crossing the sperm average path in either direction
Straightness	Measures departure of average sperm path from straight line (ratio of VSL/VAP)
Linearity	Measures departure of actual sperm track from straight line (ratio of VSL/VCL)
Elongation	Ratio (%) of head width to head length
Area	Average size in square microns of all sperm heads

(**NE**) tall fescue variety has been developed (Bouton et al., 2002) that produces little or no ergopeptines and does not reduce animal performance (Nihsen et al., 2004).

Consumption of toxic tall fescue can alter endocrine function of cattle (Browning et al., 1998a,b; Nihsen et al., 2004). Concentrations of prolactin are generally reduced in ruminants consuming toxic fescue (Schillo et al., 1988), and changes in cortisol depend on the duration of exposure to toxic tall fescue (Aldrich et al., 1993; Browning et al., 1998b; Looper et al., 2007). However, concentrations of testosterone were not altered between bulls consuming toxic fescue diets compared with bulls consuming nontoxic diets (Evans et al., 1988; Schuenemann et al., 2005a,b).

We hypothesized bulls grazing toxic EI tall fescue would have reduced sperm quality and possibly have altered endocrine function and that increased ambient temperatures would reduce sperm quality of bulls grazing toxic EI tall fescue. Therefore, the objectives were to determine effects of grazing toxic EI or NE tall fescue on sperm characteristics measured by an integrated visual optical system (**IVOS**) and serum concentrations of prolactin, cortisol, and testosterone in Brahman-influenced bulls.

MATERIALS AND METHODS

The committee for animal welfare at the USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville, AR, approved animal procedures used in this experiment.

Description of Animals, Semen Collection, and Forage Measurements

Before the experiment (May 4, 2006 to April 17, 2007), Brahman-influenced (1/8 to 3/16 Brahman) bulls were maintained on common bermudagrass [*Cynodon dactylon* (L.) Pers.] pastures overseeded with Elbon rye (*Secule cereale* L.). Bulls (n = 16; mean age = 1.1 ± 0.1 yr; mean BW = 478 ± 34 kg) were blocked by BW, scrotal circumference (**SC**), percentage motile and progressive sperm, and total sperm (millions of cells) across 3 collection dates (February 27, March 20, and April 17, 2007) before the initiation of the experiment and randomly assigned to graze toxic EI (Kentucky 31; 4 bulls/pasture; 2 pastures) or NE (MaxQ, Pennington Seed, Atlanta, GA; 4 bulls/pasture; 2 pastures) tall fescue pastures for 121 d (April 17 to August 16). Body weight was recorded and SC measured with a scrotal measuring tape (Nasco, Ft. Atkinson, WI) monthly. Semen was collected monthly at 0900 to 1030 h by electroejaculation (Electroejac IV, Ideal Instruments/Neogen Corp., Lansing, MI) and placed in a 15-mL conical centrifuge tube. Ejaculates were held in a 35.5°C water bath and evaluated within 30 min of collection. Semen was diluted 20:1 in warm Dulbecco's PBS immediately before evaluation. Sperm were evaluated for motility and morphology variables (Table 1) using a Hamilton Thorne IVOS computerized sperm analysis system (Hamilton-Thorne Biosciences, Beverly, MA) utilizing Animal Motility Software, version 12.1. Ten different fields were scanned to determine averages for all sperm variables for each monthly semen collection. Within each field, 30 video frames were captured for analysis of sperm motility. At each evaluation, semen samples for each bull were fixed using an eosin-nigrosin-based live-dead stain (Jorvet Stain, Jorgensen Laboratories, Lovelend, CO) for later evaluation of morphology. For each slide, approximately 100 sperm were evaluated for percentage live (dye exclusion) and dead, as well as for major (malformed, abnormal acrosome, proximal droplet, folded tail, midpiece defect, cratered head, pyriform head, or small/giant head) and minor (distal droplet, bent/coiled tail, headless, or tailless) defects.

Rectal temperatures (GLA M700 digital thermometer, Agricultural Electronics, San Luis Obispo, CA) of bulls were recorded in July and August before sperm collection. Maximum ambient daily temperature was recorded using a weather station (model 900, Spectrum Technologies Inc., Plainfield, IL) located 5 km from the tall fescue pastures.

Fescue pastures were fertilized with 67 kg of N/ha at the initiation of the experiment (19 April). Pastures were characterized during the experiment to determine forage availability (d 44, 79, and 118 of grazing) and concentrations of ergovaline (d 1, 44, 79, and 118 of grazing). Forage availability was evaluated using a disk meter (Bransby et al., 1977). Forage height was recorded at 50 random locations in each pasture. Regression equations of kilograms of DM/ha and disk meter height were calculated. Random grab samples (8 to 10 samples/ha) were pooled, cut into 5.1-cm pieces, and stored at −4°C. Concentrations of ergovaline were determined using HPLC as described by Hill et al. (1993). Random grab samples (10 samples/ha) were utilized for evaluating nutritive value of forage during July and August. Nutrient analysis of the forage samples was completed by the Agricultural Diagnostic Service Laboratory, University of Arkansas.

Blood Collection and Hormone Determination

Blood samples (7 mL) were obtained from bulls each month at semen collection (d 0, 30, 58, 93, and 121 of grazing). Blood samples were collected by venipuncture of the median caudal vein into vacutainers (Becton Dickinson, Franklin Lakes, NJ), allowed to clot for 24 h at 4°C, and centrifuged (1,500 × g for 25 min). Serum samples were stored at −4°C until analyses.

All hormones were quantified in single assays. Serum prolactin concentrations were determined in duplicate by double antibody RIA as described by Spoon and Hallford (1989), respectively, using primary antisera and purified standard and iodination preparations supplied by the National Hormone and Peptide Program (Torrance, CA). Intraassay CV was 7%.

Serum concentrations of cortisol and testosterone were determined in duplicate by solid phase RIA using components of commercial kits (Siemens Diagnostic, Los Angeles, CA). These kits utilize antibody-coated tube technology, and assays were performed without prior extraction of the individual hormones from serum. Cortisol and testosterone assays were validated in ruminant serum as described by Kiyma et al. (2004) and Richards et al. (1999), respectively; intraassay CV was 7 and 12%, respectively, for cortisol and testosterone.

Statistical Analyses

Pasture was the experimental unit in all statistical analyses. Dry matter availability, and CP, ADF, NDF, TDN of fescue pastures, initial and final BW, BW change, initial and final SC, SC change, and rectal temperatures (July and August) were analyzed by ANOVA utilizing the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included pasture replicate, fescue type, and the interaction. Comparisons between fescue type on SC, sperm characteristics, and concentrations of prolactin, cortisol, and testosterone were analyzed using the MIXED procedure of SAS for repeated measures (Littell et al., 1998). The model included pasture replicate, fescue type, collection date, and all interactions. Utilizing Akaike's information criterion and Schwarz' Bayesian Criterion (Littell et al., 2000), the most appropriate covariance structure for each analysis was compound symmetric. Kenward-Rogers' approximation was used for calculation of the degrees of freedom of the pooled error term. The random effect of pasture within each fescue type (specified in the SUBJECT statement) accounted for the correlations among repeated observations on the same pasture. Significance was set at $P < 0.10$ in all analyses. Concentrations of cortisol were different ($P = 0.08$) on d 0 between fescue type; therefore, concentration of cortisol on d 0 was used as a covariate in analysis of the effects of fescue type on cortisol. If the interaction of fescue type × collection date was significant ($P < 0.10$), effects of fescue type were examined within collection date using pairwise contrasts (PDIFF function of SAS).

Table 2. Mean nutrient analysis of toxic endophyte-infected (EI) or novel endophyte-infected (NE) tall fescue pastures grazed by Brahman-influenced bulls for 121 d

Item	EI	NE	SE
DM, kg/ha	5,589[a]	5,078[b]	49
Nutrient analysis (DM basis)			
CP, %	13.3[c]	15.4[d]	0.1
ADF, %	34.5	32.2	0.6
NDF, %	61.4	61.4	0.1
TDN, %	57.7	60.9	0.4
Ergovaline, mg/kg	0.60	ND[1]	0.06

[a,b]Means in a row without common superscripts differ ($P < 0.09$).
[c,d]Means in a row without common superscripts differ ($P < 0.05$).
[1]No detectable ergovaline.

RESULTS

Dry matter availability was greater ($P < 0.09$) from toxic EI pastures than NE fescue pastures (Table 2); however, DM availability was not limited for either fescue type during the current experiment. Overall CP was greater ($P < 0.05$) from NE fescue pastures than toxic EI fescue (Table 2). Percentages of ADF, NDF, and TDN were similar ($P \geq 0.12$) between fescue types (Table 2). Concentrations of ergovaline ranged from 0.39 to 0.74 mg/kg of DM (pooled SE = 0.1) for EI pastures from April to August; overall mean ergovaline was 0.60 ± 0.06 mg/kg of DM (Table 2). Ergovaline for EI pastures was 0.59 and 0.74 mg/kg in July and August, respectively. No detectable ergovaline was found in NE pastures during the experiment.

Final BW and BW change of bulls were not influenced ($P \geq 0.38$) by fescue type (Table 3); date influenced ($P < 0.0001$) BW changes (data not shown).

Table 3. Body weight, BW change, scrotal circumference (SC), SC change, rectal temperatures, and concentrations of cortisol and testosterone of Brahman-influenced bulls grazing either toxic endophyte-infected (EI) or novel endophyte-infected (NE) tall fescue for 121 d

Item	EI	NE	SE	*P*-value
Initial BW, kg	477	478	12	0.95
Final BW, kg	543	552	11	0.58
BW change, kg	66	74	6	0.38
Initial SC, cm	34.9	35.4	0.8	0.65
Final SC, cm	37.6	38.4	0.8	0.51
SC change, cm	2.7	2.9	0.6	0.79
July rectal temperature, °C	39.8	39.7	0.2	0.79
August rectal temperature, °C	40.0	40.1	0.2	0.94
Cortisol, ng/mL	15.6	17.5	0.6	0.11
Testosterone, ng/mL	12.2	10.6	1.1	0.41

Likewise, fescue type did not affect ($P \geq 0.51$) final SC or SC change during the experiment (Table 3), but date did affect ($P < 0.0001$) SC changes (data not shown). Rectal temperatures recorded in July and August did not differ ($P \geq 0.79$) between fescue types (Table 3).

Sperm Characteristics

There were minor differences ($P < 0.06$) between fescue types for sperm characteristics in April, May, or June. Track velocity of sperm over the actual point-to-point track were slower ($P = 0.06$) in bulls grazing toxic EI (159 ± 7 μm/sec) fescue than bulls grazing NE (196 ± 7 μm/sec) fescue. Lateral amplitude of head oscillation as the sperm swims was less ($P < 0.01$) in toxic EI (6.1 ± 0.1 μm) bulls compared with NE (6.6 ± 0.1 μm) fescue bulls in April, May, and June. There was not ($P > 0.10$) a date × fescue type interaction for sperm characteristics in April, May, or June.

Fescue type did alter ($P < 0.10$) sperm characteristics in July (mean maximum daily ambient temperature = 33.6°C) and August (mean maximum daily ambient temperature = 39.7°C). Percentages of motile and progressive sperm were greater ($P < 0.06$) in NE bulls compared with EI bulls; percentage of rapid sperm ($P = 0.04$) was greater from bulls grazing NE than EI bulls (Figure 1). Percentage of medium progressive sperm tended ($P = 0.10$) to be greater from bulls grazing toxic EI fescue than bulls grazing NE fescue. Date (July and August) or date × fescue type interaction did not alter ($P > 0.10$) percentages of motile, progressive, or medium progressive sperm. A fescue × date interaction influenced ($P = 0.04$) percentage of slow progressive sperm; however, within date comparisons of slow progressive sperm were not different ($P \geq 0.25$) between fescue type (data not shown). Date (July or August), fescue type or the interaction between date and fescue type did not influence ($P > 0.10$) sperm concentration (millions of cells/mL), percentages of live sperm, or sperm with major or minor defects (Table 4).

Average velocity of the smoothed sperm path and progressive velocity in a straight line from the beginning to the end of the sperm track were slower ($P \leq 0.09$) in EI bulls than NE bulls and were slower ($P = 0.04$) in August compared with July (Table 5). Track velocity of sperm over the actual point-to-point track was slower ($P = 0.02$) in August compared with July (Table 5). Lateral amplitude of head oscillation as the sperm swims was less ($P < 0.06$) in August than July (Table 5). Beat frequency, which is the frequency of the sperm head crossing the sperm average path in either direction, was greater ($P = 0.09$) in July than August; fescue type did not affect beat frequency ($P = 0.53$; Table 5). Sperm characteristics of straightness [ratio of progressive velocity (**VSL**)/path velocity (**VAP**)], linearity [ratio of VSL/track speed (**VCL**)], elongation (ratio of head width to head length), and area (average size of sperm heads) of sperm were not influenced by fescue type ($P \geq 0.27$), collection date ($P \geq 0.17$), or the interaction ($P \geq 0.17$).

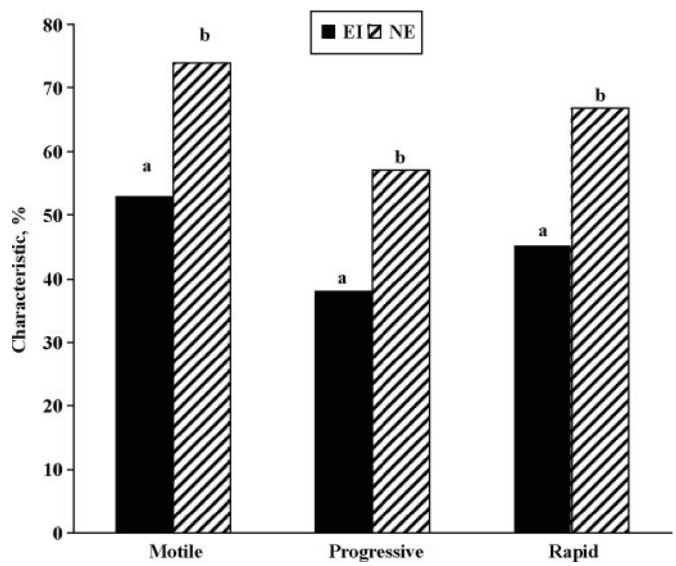

Figure 1. Mean percentages of semen characteristics of Brahman-influenced bulls grazing either toxic endophyte-infected (EI) or novel endophyte-infected (NE) tall fescue in July and August. Characteristics are defined in Table 1. Fescue type, $^{a,b}P < 0.06$; collection date and collection date × fescue type, $P > 0.10$. Pooled SE = 3.8, 3.4, and 3.0 for motile, progressive, and rapid sperm, respectively.

Table 4. Influence of toxic endophyte-infected (EI) or novel endophyte-infected (NE) tall fescue and collection date on semen characteristics of Brahman-influenced bulls

	Fescue type			Collection date			*P*-value		
Item	EI	NE	SE	July	August	SE	Fescue	Date	Fescue × date
Sperm concentration, millions of cells/mL	758	607	298	589	776	267	0.76	0.57	0.51
Live sperm cells, %	67.4	80.1	5.4	72.9	74.6	4.8	0.24	0.77	0.99
Major[1] defects, %	11.3	10.9	4.0	11.1	11.2	3.1	0.95	0.96	0.84
Minor[2] defects, %	17.7	13.5	6.6	14.6	16.7	5.2	0.69	0.64	0.80

[1]Major defects = malformed, abnormal acrosome, proximal droplet, folded tail, midpiece defect, cratered head, pyriform head, or small/giant head.

[2]Minor defects = distal droplet, bent/coiled tail, headless, or tailless defects.

Endocrine Factors

Mean concentrations of prolactin were influenced ($P < 0.08$) by a fescue type × date interaction. Concentrations of prolactin were similar between bulls grazing EI and NE fescue in April; however, prolactin was less in EI bulls than NE bulls May through August (Figure 2). Concentrations of cortisol were not influenced ($P > 0.10$) by date, fescue type, or the interaction (Table 3). Similarly, concentrations of testosterone were not affected by fescue type ($P = 0.41$; Table 3); however, testosterone was influenced ($P = 0.09$) by date. Concentrations of testosterone were variable during the experiment and greatest in June (14.5 ± 1.5 ng/mL) and August (12.5 ± 1.5 ng/mL) and less in May (10.6 ± 1.5 ng/mL), April (10.2 ± 1.5 ng/mL), and July (9.1 ± 1.5 ng/mL).

DISCUSSION

Studies comparing the effects of toxic and nonergot alkaloid-producing tall fescue varieties on bull reproductive performance with an IVOS are nonexistent. Several sperm characteristics as measured by IVOS were affected by fescue type in the current experiment. These results are in contrast to several studies that report no differences in sperm motility and morphology in bulls consuming toxic EI fescue diets or nontoxic diets (Evans et al., 1988; Schuenemann et al., 2005a,b). Laboratory methodologies of sperm analyses may explain difference among studies. Previous reports utilized subjective procedures, whereas an IVOS analysis was used in the current experiment. Farrell et al. (1998) reported that computer-assisted sperm analysis was highly repeatable (repeatability = 0.99) and provided a better estimate of sperm motility than traditional subjective procedures. Many of the IVOS measurements are not possible in routine evaluations.

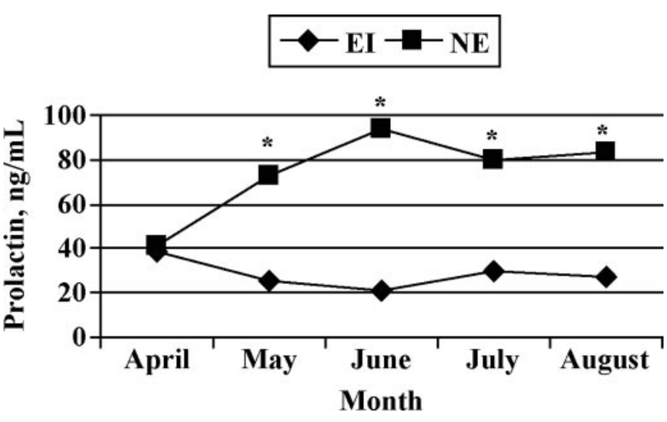

Figure 2. Concentrations of prolactin of Brahman-influenced bulls grazing either toxic endophyte-infected (EI) or novel endophyte-infected (NE) tall fescue for 121 d. Fescue type × date interaction (*$P < 0.08$); pooled SE = 14.0.

Sperm motility and percentage of progressive sperm were reduced in bulls grazing toxic EI fescue compared with bulls grazing NE fescue in July and August. Angus bulls fed toxic EI fescue seed diets tended to have reduced motility compared with bulls consuming a nontoxic diet during the last 2 wk of a 60-d study (Jones et al., 2004). Wang (2006) reported ergot alkaloids commonly found in toxic EI fescue plants reduced sperm motility in a concentration-dependent manner. Percentages of intact acrosomes and postthaw motility were decreased when ergonovine was added as an extender for sperm cryopreservation (Gallagher and Senger, 1989). In humans, vaginal treatment with bromocriptine, an ergoline derivative, decreased sperm motility by 31% (Chenette et al., 1991). In the current experiment, percentage of rapid progressive sperm was decreased in EI compared with NE bulls in July and August. Schuenemann et al. (2005a) reported that although sperm motility and morphology did not differ between bulls grazing toxic EI or NE fescue pastures from November to June, potential of sperm to fertilize oocytes was reduced from bulls grazing toxic EI fescue. Similarly, we did not detect differences in sperm motility in May and June. Changes in sperm quality between bulls grazing toxic EI or NE fescue pastures were detected only after ambient temperatures increased in July and August. Differences between Schuenemann et al. (2005a) and the current study also may be attributed to laboratory methodologies; Schuenemann et al. (2005a) used subjective procedures, whereas an IVOS analysis was used in the current experiment. Sperm motility of Holstein bulls ranged from 62 to 69% with subjective procedures, whereas the range was 52 to 82% for computer-assisted sperm analysis; repeatability was 0.99 for computer-assisted sperm analysis (Farrell et

Table 5. Influence of toxic endophyte-infected (EI) or novel endophyte-infected (NE) tall fescue and collection date on semen characteristics of Brahman-influenced bulls

	Fescue type			Collection date			*P*-value		
Item[1]	EI	NE	SE	July	August	SE	Fescue	Date	Fescue × date
VAP, μm/sec	97.5	123.9	5.4	122.7	98.7	6.7	0.07	0.04	0.78
VSL, μm/sec	82.3	105.6	5.2	105.2	82.7	6.4	0.09	0.04	0.78
VCL, μm/sec	165.1	204.7	11.0	205.4	164.5	11.3	0.12	0.02	0.70
ALH, μm	6.6	7.8	0.5	7.7	6.6	0.5	0.25	0.06	0.97
BCF, Hz	27.3	28.7	1.4	30.0	26.0	1.5	0.53	0.09	0.96

[1]VAP = path velocity, average velocity of the smoothed cell path; VSL = progressive velocity, average velocity measured in a straight line from beginning to end; VCL = track speed, average velocity measured over the actual point-to-point track; ALH = lateral amplitude, mean width of head oscillation as the sperm swims; BCF = frequency of sperm head crossing the sperm average path in either direction.

al., 1998). In humans, sperm motility and concentration, as measured by computer-assisted semen analysis, were more accurate in identifying infertile men than sperm morphology (Nallella et al., 2006). Although motility was reduced in semen collected from bulls grazing toxic EI fescue in the current experiment, this may not be an indication of reduced fertility. Fertility was not highly correlated with motility once a certain motility threshold was obtained in boars (Flowers, 1997). Highly motile sperm cells may not always result in increased fertility (Hall, 1981). It appears sperm motility is reduced and percentages of progressive and rapid sperm are decreased in bulls grazing toxic EI fescue pastures; however, consequences of decreased motility on bull fertility should be the basis of future studies.

Average velocities (VAP and VSL) of sperm were slower in EI bulls than NE bulls. Kato et al. (2001) concluded that using both the percentage of progressive sperm and sperm velocity parameters could detect adverse effects on rat sperm motion. Correlations between fertility of dairy bulls and computer-assisted sperm analysis variables ranged from 0.63 to 0.67 when percent progressive and velocity measures (VAP, VSL, and VCL) were included in the model (Farrell et al., 1998). Sperm collected in the current experiment had slower path velocities, less lateral amplitude of the sperm head, and slower beat frequency of the sperm head during August compared with July, suggesting toxic effects of EI consumption may be exacerbated by increased ambient temperatures. Exposure of Angus bulls to elevated ambient temperatures (35°C for 8 h and 31°C for 16 h) resulted in reduced percentage of motile sperm and an increased percentage of abnormal sperm (Meyerhoeffer et al., 1985). Mean maximum daily ambient temperature for July and August semen collection dates were 33.6 and 39.7°C, respectively. Speed of sperm was slower in EI bulls than NE and during warmer weather in the current experiment. Consumption of toxic EI fescue may be detrimental to the velocity of sperm, which along with the percentage of progressive sperm, has been associated with bull fertility (Farrell et al., 1998). Increased ambient temperatures may further intensify the negative effects of the consumption of toxic EI fescue.

Concentrations of ergovaline averaged 0.60 mg/kg of DM for EI pastures from April to August. Ergovaline is the primary ergopeptine found in toxic fescue (Lyons et al., 1986) and is considered to play a role in fescue toxicity (Porter, 1995); however, other ergot alkaloids also are likely involved in causing fescue toxicosis (Hill, 2005). Although threshold concentrations of ergovaline that induce fescue toxicosis are not fully established, BW gain of beef steers was not affected by fescue straw containing up to 0.475 mg/kg of DM as ergovaline consumed during winter (Stamm et al., 1994). Toxic fescue seed diets with 0.75 mg/kg of DM of ergovaline resulted in reduced DMI, increased urinary alkaloids, and tended to decrease prolactin in ewes after 7 d (Looper et al., 2007). Concentrations of ergovaline in July and August in the current experiment were similar to published values of ergovaline capable of inducing fescue toxicosis in cattle and sheep (Aiken et al., 2006; Looper et al., 2007). Dry matter availability was less in NE fescue pastures than toxic EI fescue pastures; however, DM availability was not limited for either fescue type at anytime during the experiment. Crude protein was less in toxic EI fescue pastures than NE fescue pastures during the experiment; all other nutritive components measured were similar between fescue types. Both fescue types exceeded the nutrient requirements of growing bulls (NRC, 1984). Increased CP of NE fescue pastures did not result in improved BW gain of bulls grazing NE fescue.

Bulls grazing toxic EI fescue pastures had decreased concentrations of prolactin from May throughout the duration of the current experiment compared with bulls grazing NE fescue. Reduced concentrations of prolactin are frequently used as a physiological indicator of fescue toxicosis in ruminants (Porter and Thompson, 1992; Aiken et al., 2006). We recently reported that ewes fed increased ergovaline (0.75 mg/kg) fescue seed diets tended to have reduced concentrations of prolactin compared with ewes fed decreased ergovaline (0.11 mg/kg) fescue seed diets after 7 d of diet consumption (Looper et al., 2007). In the current experiment, ergovaline values and reduced prolactin from bulls consuming toxic EI fescue provided confirmation that ergot alkaloids in toxic EI fescue pastures were adequate to induce physiological changes associated with fescue toxicosis.

Change in BW of bulls was not altered by fescue type in the current experiment. Average daily gain was similar from crossbred Angus bulls fed a corn:corn silage diet with or without 40 μg/kg of BW of ergotamine tartrate for 224 d (Schuenemann et al., 2005b). However, Angus and Gelbvieh bulls grazing NE fescue pastures for 224 d had greater ADG than bulls grazing toxic EI fescue pastures (Schuenemann et al., 2005a). Typically, decreased ADG associated with fescue toxicosis is primarily due to reduced DMI (Paterson et al., 1995; Looper et al., 2007). Brown et al. (1997) suggested Brahman-influenced cattle appear to be more tolerant of the negative effects of toxic fescue than Angus cattle and may partially explain why no differences in BW gain were observed in the current experiment.

Rectal temperatures in July and August did not differ between EI and NE bulls. Body temperature is usually increased in ruminants consuming toxic fescue (Hoveland et al., 1983; Nihsen et al., 2004); however, others have reported no effect of toxic fescue on body temperatures (Stamm et al., 1994). It has been suggested that animals may become acclimated to the toxic effects of fescue after prolonged exposure (Al-Haidary et al., 2001). Bulls would have been exposed to the toxic effects of EI fescue for 94 and 121 d, respectively, for the July and August rectal temperature measurements. It is possible that bulls grazing EI pastures may have become acclimated to the toxic fescue during the grazing season (Spiers et al., 2005). A further explanation of the current results may be the increased heat tolerance of Brahman-influenced cattle (Brown et al., 1997).

Final SC as well as SC change during the current experiment was not influenced by fescue type. Development of Holstein bulls on toxic fescue hay from 2 to 13 mo of age did not impact SC (Evans et al., 1988). Scrotal circumference of bulls was not affected by administration of ergotamine tartrate (Schuenemann et al., 2005b) or grazing of toxic EI fescue pastures (Schuenemann et al., 2005a). Jones et al. (2004) found mean SC of Angus bulls was decreased in bulls consuming toxic EI seed diets during July through mid-September in southern Illinois compared with bulls consuming nontoxic seed diets for 60 d. However, during the last 10 d of that study, SC did not differ between fescue seed diets. Mean concentrations of ergovaline (1.0 mg/kg of DM) in the Jones et al. (2004) study were almost 1.6 times the mean ergovaline values (0.60 mg/kg of DM) in the current experiment and may have contributed to inconsistencies between studies.

Concentrations of cortisol were not affected by fescue type in the current experiment. Studies investigating cortisol response to toxic EI fescue have not been consistent and may depend on the duration of exposure. Browning et al. (1998b) reported cortisol was increased in steers and cows 3 to 4 h after infusion of the ergot alkaloid ergotamine. However, when samples were collected daily, concentrations of cortisol in heifers fed EI fescue diets for 20 d were similar to cortisol in animals consuming endophyte-free fescue seed diets (Aldrich et al., 1993). Further, concentrations of cortisol were not different between ewes fed increased ergovaline (0.75 mg/kg) fescue seed diets for 7 d and ewes fed decreased ergovaline (0.11 mg/kg) fescue seed diets (Looper et al., 2007). These studies and others (Al-Haidary et al., 2001) suggest ruminants may become acclimated to the toxic effects of EI fescue, and cortisol may not mediate the detrimental effects of toxic EI fescue in the animal.

Similar to cortisol, concentrations of testosterone were similar between toxic EI and NE bulls. These results are in agreement with several reports indicating toxic EI fescue does not impair circulating concentrations of testosterone (Evans et al., 1988; Schuenemann et al., 2005a,b). Lunstra et al. (1978) reported a positive correlation between SC and concentrations of testosterone. Lack of an effect of fescue type on SC is consistent with no change in testosterone concentrations.

Semen from Brahman-influenced bulls grazing toxic EI fescue pastures had reduced motility and morphology during periods of increased maximum ambient temperatures compared with bulls grazing NE fescue pastures. Concentrations of prolactin were reduced more in bulls grazing EI than NE bulls; physiological stress on reproductive performance of bulls induced by consumption of toxic EI fescue did not appear to be mediated through circulating concentrations of cortisol or testosterone. The relationship of reduced sperm quality and its effects on fertility from bulls consuming toxic EI fescue should be the basis of future studies.

LITERATURE CITED

Aiken, G. E., M. L. Looper, S. F. Tabler, D. K. Brauer, J. R. Strickland, and F. N. Schrick. 2006. Influence of stocking rate and steroidal implants on growth rate of steers grazing toxic tall fescue and subsequent physiological responses. J. Anim. Sci. 84:1626–1632.

Aldrich, C. G., J. A. Paterson, J. L. Tate, and M. S. Kerley. 1993. The effects of endophyte-infected tall fescue consumption on diet utilization and thermal regulation in cattle. J. Anim. Sci. 71:164–170.

Al-Haidary, A., D. E. Spiers, G. E. Rottinghaus, G. B. Garner, and M. R. Ellersieck. 2001. Thermoregulatory ability of beef heifers following intake of endophyte-infected tall fescue during controlled heat challenge. J. Anim. Sci. 79:1780–1788.

Bouton, J. H., G. C. M. Latch, N. S. Hill, C. S. Hoveland, M. A. McCann, R. H. Watson, J. A. Parish, L. L. Hawkins, and F. N. Thompson. 2002. Reinfection of tall fescue cultivars with nonergot alkaloid-producing endophytes. Agron. J. 94:567–574.

Bransby, D. I., A. G. Matches, and G. F. Krause. 1977. Disk meter for rapid estimation of herbage yield in grazing trials. Agron. J. 69:393–396.

Brown, M. A., A. H. Brown Jr., W. G. Jackson, and J. R. Miesner. 1997. Genotype × environment interactions in Angus, Brahman, and reciprocal cross cows and their calves grazing common bermudagrass and endophyte-infected tall fescue pastures. J. Anim. Sci. 75:920–925.

Browning, R. Jr., M. L. Leite-Browning, H. M. Smith, and T. Wakefield. 1998b. Effect of ergotamine and ergonovine on plasma concentrations of thyroid hormones and cortisol in cattle. J. Anim. Sci. 76:1644–1650.

Browning, R. Jr., F. N. Schrick, F. N. Thompson, and T. Wakefield. 1998a. Reproductive hormonal responses to ergotamine and ergonovine in cows during the luteal phase of the estrous cycle. J. Anim. Sci. 76:1448–1454.

Burke, J. M., D. E. Spiers, F. N. Kojima, G. A. Perry, B. E. Salfen, S. L. Wood, D. J. Patterson, M. F. Smith, M. C. Lucy, W. G. Jackson, and E. L. Piper. 2001. Interaction of endophyte-infected fescue and heat stress on ovarian function in the beef heifer. Biol. Reprod. 65:260–268.

Chenette, P. E., M. S. Siegel, M. Vermesh, and O. A. Kletzky. 1991. Effect of bromocriptine on sperm function *in vitro* and *in vivo*. Obstet. Gynecol. 77:935–938.

Evans, K. L., P. M. Zavos, R. W. Hemken, and J. A. Jackson Jr. 1988. Effects of feeding endophyte-infected (*Acremonium coenophialum*) KY-31 fescue hay on the reproductive development of Holstein bulls. Theriogenology 30:169–179.

Farrell, P. B., G. A. Presicce, C. C. Brockett, and R. H. Foote. 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Theriogenology 49:871–879.

Flowers, W. L. 1997. Management of boars for efficient semen production. J. Reprod. Fertil. 52:67–78.

Gallagher, G. R., and P. L. Senger. 1989. Effect of phenylephrine, ergonovine, oxytocin and norepinephrine as an extender ingredient on viability of bovine spermatozoa. J. Anim. Sci. 67:1573–1576.

Hall, J. L. 1981. Relationship between semen quality and human sperm penetration of zona-free hamster ova. Fertil. Steril. 35:457–463.

Hill, N. S. 2005. Absorption of ergot alkaloids in the ruminant. Pages 271–290 in Neotyphodium in Cool-Season Grasses. C. Roberts, C. West, and D. Spiers, ed. Blackwell Publishing, Ames, IA.

Hill, N. S., G. E. Rottinghaus, C. S. Agee, and L. M. Schultz. 1993. Simplified sample preparation for HPLC analysis and ergovaline in tall fescue. Crop Sci. 33:331–333.

Hoveland, C. S., S. P. Schmidt, C. C. King Jr., J. W. Odom, E. M. Clark, J. A. McGuire, L. A. Smith, H. W. Grimes, and J. L. Holliman. 1983. Steer performance and association of Acremonium coenophialum fungal endophyte on tall fescue pasture. Agron. J. 75:821–824.

Jones, K. L., S. S. King, K. E. Griswold, D. Cazac, and D. L. Cross. 2003. Domperidone can ameliorate deleterious reproductive effects and reduced weight gains associated with fescue toxicosis in heifers. J. Anim. Sci. 81:2568–2574.

Jones, K. L., C. R. McCleary, S. S. King, G. A. Apgar, and K. E. Griswold. 2004. Case Study: Consumption of toxic fescue impairs bull reproductive parameters. Prof. Anim. Sci. 20:437–442.

Kato, M., K. Fukunishi, S. Ikegawa, H. Higuchi, M. Sato, M. Horimoto, and S. Ito. 2001. Overview of studies on rat sperm motion analysis using a Hamilton-Thorne sperm analyzer: Collaborative working study. J. Toxicol. Sci. 26:285–297.

Kiyma, Z., B. M. Alexander, E. A. Van Kirk, W. J. Murdoch, D. M. Hallford, and G. E. Moss. 2004. Effects of feed restriction on reproductive and metabolic hormones in ewes. J. Anim. Sci. 82:2548–2557.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216–1231.

Littell, R. C., J. Pendergast, and R. Natarajan. 2000. Modelling covariance structure in the analysis of repeated measures data. Stat. Med. 19:1793–1819.

Looper, M. L., T. S. Edrington, R. Flores, J. M. Burke, T. R. Callaway, G. E. Aiken, F. N. Schrick, and C. F. Rosenkrans Jr. 2007. Influence of dietary endophyte (*Neotyphodium coenophialum*)-infected tall fescue (*Festuca arundinacea*) seed on fecal shedding of antibiotic resistance-selected *Escherichia coli* O157:H7 in ewes. J. Anim. Sci. 85:1102–1108.

Lunstra, D. D., J. J. Ford, and S. E. Echternkamp. 1978. Puberty in beef bulls: Hormone concentrations, growth, testicular development, sperm production and sexual aggressiveness in bulls of different breeds. J. Anim. Sci. 46:1054–1062.

Lyons, P. C., R. D. Plattner, and C. W. Bacon. 1986. Occurrence of peptide and clavinet ergot alkaloids in tall fescue. Science 232:487–489.

Meyerhoeffer, D. C., R. P. Wettemann, S. W. Coleman, and M. E. Wells. 1985. Reproductive criteria of beef bulls during and after exposure to increased ambient temperature. J. Anim. Sci. 60:352–357.

Nallella, K. P., R. K. Sharma, N. Aziz, and A. Agarwal. 2006. Significance of sperm characteristics in the evaluation of male infertility. Fertil. Steril. 85:629–634.

Nihsen, M. E., E. L. Piper, C. P. West, R. J. Crawford Jr., T. M. Denard, Z. B. Johnson, C. A. Roberts, D. A. Spiers, and C. F. Rosenkrans Jr. 2004. Growth rate and physiology of steers grazing tall fescue inoculated with novel endophytes. J. Anim. Sci. 82:878–883.

NRC. 1984. Nutrient Requirements of Beef Cattle. Natl. Acad. Sci., Washington, DC.

Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. 1995. The effects of fescue toxicosis on beef cattle productivity. J. Anim. Sci. 73:889–898.

Porter, J. K. 1995. Analysis of endophyte toxins: Fescue and other grasses toxic to livestock. J. Anim. Sci. 73:871–880.

Porter, J. K., and F. N. Thompson Jr. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70:1594–1603.

Richards, J. B., D. M. Hallford, and G. C. Duff. 1999. Serum luteinizing hormone, testosterone and thyroxine and growth responses of ram lambs fed locoweed (Oxytropis sericea) and treated with vitamin E/selenium. Theriogenology 52:1055–1066.

Schillo, K. K., L. S. Leshin, J. A. Boling, and N. Gay. 1988. Effects of endophyte-infected fescue on concentrations of prolactin in blood sera and the anterior pituitary and concentrations of dopamine and dopamine metabolites in brains of steers. J. Anim. Sci. 66:713–718.

Schuenemann, G. M., J. L. Edwards, M. D. Davis, H. E. Blackmon, F. N. Scenna, N. R. Rohrbach, A. M. Saxton, H. S. Adair, F. M. Hopkins, J. C. Waller, and F. N. Schrick. 2005b. Effects of administration of ergotamine tartrate on fertility of yearling beef bulls. Theriogenology 63:1407–1418.

Schuenemann, G. M., J. L. Edwards, F. M. Hopkins, N. R. Rohrbach, H. S. Adair, F. N. Scenna, J. C. Waller, J. W. Oliver, A. M. Saxton, and F. N. Schrick. 2005a. Fertility aspects in yearling bulls grazing endophyte-infected tall fescue pastures. Reprod. Fertil. Dev. 17:479–486.

Spiers, D. E., T. J. Evans, and G. E. Rottinghaus. 2005. Interaction between thermal stress and fescue toxicosis: Animal models and new perspectives. Page 248 in Neotyphodium in Cool-Season Grasses. Roberts, West, and Spiers, ed. Blackwell Publ., Ames, IA.

Spoon, R. A., and D. M. Hallford. 1989. Growth response, endocrine profiles and reproductive performance of fine-wool ewe lambs treated with ovine prolactin before breeding. Theriogenology 32:45–53.

Stamm, M. M., T. Delcurto, M. R. Horney, S. D. Brandyberry, and R. K. Barton. 1994. Influence of alkaloid concentration of tall fescue straw on the nutrition, physiology, and subsequent performance of beef steers. J. Anim. Sci. 72:1068–1075.

Wang, H. 2006. Toxic effects of ergot alkaloids on bovine sperm motility and possible mechanisms of toxicity. PhD Diss. Univ. Arkansas, Fayetteville.